



Research Article

THERAPEUTIC EFFICACY OF *Pisonia alba* AGAINST ATRAZINE TOXICITY ON BIOCHEMICAL PARAMETERS IN THE LIVER TISSUE OF ALBINO WISTER RAT *Rattus norvegicus*

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Abstract

The present study was undertaken to evaluate the hepatoprotective effects of *P. alba* against the toxicity effects of herbicide atrazine on biochemical enzymes activity in the Albino Wister rat *Rattus norvegicus*. In the present experimental study, *Rattus norvegicus* were administered to sub-lethal dose of atrazine (20 mg/l of atrazine) for 28 days. The variation of biochemical parameter like protein and glycogen levels decreased in the liver tissue of atrazine treated rat, simultaneously the glucose and amino acid level was increased compared to the control. During the treatment of *Pisonia alba* against atrazine administered rat were restored near normal level (Group III and IV). The observed results were discussed in detail in this present research paper.

Article History

Received : 20.11.2015

Revised : 05.12.2015

Accepted : 16.12.2015

Key words: Atrazine, *Rattus norvegicus*, *Pisonia alba*, Biochemical enzymes and Liver tissue.

1. Introduction

Atrazine (IUPAC: 6-chloro-N2-ethyl-N4-isopropyl-1, 3, 5-triazine-2,4-diamine) is a triazine herbicide widely used in the production of corn, sugar cane and sorghum. It is commonly used in non-EU countries and it is one of the most used pesticides in the US (US EPA 2003). Approximately, 1 to 6 % of the applied herbicides are released to the aquatic environment. Aged and persistent herbicides can become recalcitrant due to increased sorption and decreased bioavailability over time (Felsot *et al.*, 1997). The increasing interest of biological effects of atrazine is related to the detection of this compound in groundwater, surface water and in drinking water (WHO-1996).

The experimental studies had provided sufficient evidence to classify atrazine as an endocrine disruptor and reproductive toxicant (Foradori *et al.*, 2010; Abarikwu *et al.*, 2010).

Pisonia grandis (Synonym: *Pisonia alba*, *Pisonia morindifolia*) commonly known as Leechikottaikerai in Tamil, Velatisalet in Hindi (Khare, 2007). The plant *Pisonia grandis*, belonging to the family Nyctaginaceae, is an evergreen glabrous garden tree with young shoots are minutely puberulous. It is native of Hawaii Island and naturalized throughout India. In the alternative system of medicine, *Pisonia grandis* leaves are used as analgesic, anti-inflammatory, diuretic (Radha *et al.*, 2008), hypoglycemic agent (Sunil *et al.*, 2010), antifungal (Shubashini and Poongothai, 2010). It is also used in the treatment of ulcer, dysentery

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and snake bite. The leaves are edible and mostly used to treat wound healing, rheumatism and arthritis (Prabu *et al.*, 2008). Leaves consumed as salad and also fed to cattle (Chatterjee and Prakash, 1997).

2. Materials and Methods

Experimental animal

Adult male albino Wistar rat (*Rattus norvegicus*) weighing (150 - 200 g) were obtained from Central Animal House, Rajah Muthiah Medical College, (Reg No. 160/1999/CPCSEA, Proposal number: 1096/2014), Annamalai University were used for the present investigation. The study protocol was approved by the Ethics Committee on Animal Experiment, Faculty of Science, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

Experimental chemical

Experimental chemical atrazine was purchased from (TATA Atrataf 50 % WP) manufactured by Rallis India Limited, Mumbai.

Preparation of Ethanolic extract

The dried powder was extracted (*Pisonia alba*) in a Soxhlet apparatus using ethanol at a temperature range of 55 °C to 60 °C. The filtrate was evaporated to dryness at reduced pressure in a vacuum evaporator.

Preparation of samples

A 10 % (w/v) tissue homogenate was prepared in 50 mM Tris HCl (pH 7.4) using a homogenizer. Post-mitochondrial supernatant (PMS) was prepared by centrifuging the homogenate at 10,000 rpm for 10 min at 40 °C. Various biochemical parameters were assayed in the homogenate and post mitochondrial supernatant of rat liver tissue.

Biochemical analysis in liver

Liver tissue was dissected out and homogenized (10 × volumes) in potassium phosphate buffer (0.1 M, pH 7.0) and centrifuged (20 min, 13000 g, 4 °C) for biochemical analysis. The protein content of the sample was determined according to the method of Lowry *et al.* (1951) using crystalline bovine serum albumin standard.

The glycogen and glucose was estimated by the method of Adrienne and Kits Van Hejningen (1954). The free amino acids were determined by the method of Moore and Stein (1954).

Experimental design

A total of 24 animals will be divided into 4 groups of 6 in each group.

Group 1: Control animals

Group 2: Atrazine alone (0.25 mg/kg bw)

Group 3: Atrazine (0.25mg/kg bw) + *Pisonia alba* (1 g/kg bw)

Group 4: *Pisona alba* (1 g/kg bw)

Statistical Analysis

Results were expressed as mean ± S.E. Statistical analysis was performed using Student's t-test and p-values < 0.05; P<0.01 were considered statistically significant.

3. Results

In the present study, it was observed that the liver tissue of biochemical parameters such as glucose and amino acid levels are increased significantly in the treated Group II. At the end of 28 days glucose and amino acid levels are increased when compared to control Group I. In the Group III and IV, glucose and amino acid levels are near to normal when compared to Group II. In the Group IV, glucose levels are decreased significantly at 28 days compared to Group II and which was near to control Group I. Protein and glycogen levels were significantly decreased in the Group II when compared to control Group I. In the Group III and IV, protein and glycogen level was increased compared to Group II. In the Group IV, protein and glycogen level increased significantly at 28 days compared to Group II and which was near to control Group I.

Table – 1: Variations of Protein (mg/g wet wt. of tissue) in the liver tissue of Albino Wister rat *Rattus norvegicus* administered to atrazine followed by the extract of *Pisonia alba* exposed to 28 days

Groups		Treatment Duration				
		1	7	14	21	28
Protein	Group - I Control	9.323 ± 0.45	9.342 ± 0.41	9.365 ± 0.44	9.415 ± 0.31	9.436 ± 0.36
	Group - II Atrazine	7.243 ± 0.35 ^{NS} -22.310	7.214 ± 0.14 ^{NS} -22.778	7.119 ± 0.34 ^{NS} -22.982	6.839 ± 0.40 ^{**} -27.360	6.805 ± 0.36 ^{**} -27.882
	Group - III Atrazine + <i>P. alba</i>	8.463 ± 0.31 ^{**} 16.843	8.629 ± 0.23 ^{**} 19.614	8.834 ± 0.44 ^{NS} 24.090	8.915 ± 0.14 ^{NS} 30.355	8.948 ± 0.26 ^{NS} 31.491
	Group - IV <i>P. alba</i>	9.436 ± 0.18 ^{NS} 0.2467	9.425 ± 0.42 ^{NS} 0.888	9.618 ± 0.34 ^{NS} 2.7015	9.824 ± 0.24 [*] 4.344	9.915 ± 0.36 ^{NS} 5.076

(Values are mean ± S.E - Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at ** P<0.01 levels. (+,-), NS- Non-significant.

Table – 2: Variations of Amino acid levels (mg/g wet wt. of tissue) in the liver tissue of albino Wister rat *Rattus norvegicus* administered to atrazine followed by the extract of *Pisonia alba* exposed to 28 days

Groups		Treatment Duration				
		1	7	14	21	28
Amino acid	Group - I Control	89.36 ± 3.32	89.52 ± 3.18	89.70 ± 4.12	39.75 ± 4.38	89.91 ± 4.25
	Group - II Atrazine	108.14. ±6.19* 21.016	110.25 ± 6.42 * 23.156	114.43 ± 6.51 ^{**} 27.469	121.45 ±7.34 ^{**} 35.320	124.36 ± 5.15 ^{**} 38.316
	Group - III Atrazine + <i>Pisonia alba</i>	110.54 ± 4.61 ^{NS} 2.219	101.18 ± 5.34* 8.226	96.34 ± 4.13* -15.742	94.24 ± 4.34* -22.404	93.38 ± 3.36* -24.911
	Group - IV <i>Pisonia alba</i>	90.18 ± 3.12 ^{**} 0.917	90.24 ± 3.18 ^{NS} 0.804	90.48 ± 3.42 ^{NS} 0.869	90.65 ± 3.19 ^{NS} 1.002	90.84 ± 3.84 ^{NS} 1.034

Values are mean ± S.E - Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at ** P < 0.01 levels. (+,-), NS- Non-significant.

Table – 3: Variations of Glucose levels (mg/g wet wt. of tissue) in the liver tissue of Albino Wister *Rattus norvegicus* administered to atrazine followed by the extract of *Pisonia alba* exposed to 28 days

Groups		Treatment Duration				
		1	7	14	21	28
Glucose	Group-I Control	86.342 ± 3.15	86.639 ± 4.25	87.213 ± 3.18	87.815 ± 2.15	87.925 ± 3.25
	Group-II atrazine	131.421 ± 4.18 52.209	135.364 ± 4.34** 56.293	139.242 ± 5.42 59.675	142.182 ± 5.39 61.910	149.283 ± 5.24 69.784
	Group-III atrazine+ <i>Pisonia alba</i>	101.242 ± 6.12** -22.963	98.287 ± 5.34** -27.395	98.415 ± 6.12 ^{NS} -46.812	97.242 ± 4.24** -31.607	96.184 ± 3.19** -35.569
	Group-IV <i>P. Alba</i>	94.242 ± 5.34* 9.149	94.418 ± 4.25* 8.978	95.245 ± 4.39** 9.209	90.182 ± 4.28 ^{NS} 2.695	90.215 ± 4.34 ^{NS} 2.604

Values are mean ± S.E - Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at **P<0.01 levels. (+,-), NS- Non-significant.

Table – 4: Variations of Glycogen levels (mg/g wet wt. of tissue) in the liver tissue of albino Wister *Rattus norvegicus* administered to atrazine followed by the extract of *Pisonia alba* exposed to 28 days

Groups		Treatment duration				
		1	7	14	21	28
Glycogen	Group - I Control	19.27 ± 0.62	19.28 ± 0.18	19.34 ± 0.54	19.49 ± 1.18	19.68 ± 1.25
	Group - II Atrazine	15.39 ± 0.62** -20.134	15.24 ± 0.82* -20.954	14.18 ± 1.31** -26.680	14.05 ± 0.82** -27.911	13.19 ± 0.39** -32.97
	Group - III Atrazine + <i>Pisonia alba</i>	16.36 ± 0.53 ^{NS} 16.302	16.43 ± 1.3 ^{NS} 6 7.808	17.24 ± 1.21* 21.779	18.39 ± 0.69** 30.881	18.83 ± 0.84** 42.75
	Group - IV <i>Pisonia alba</i>	18.15 ± 1.25 ^{NS} -5.812	18.43 ± 1.15 ^{NS} -4.408	18.68 ± 1.31 ^{NS} -3.412	19.08 ± 1.25 ^{NS} -2.10	19.42 ± 1.15 ^{NS} -1.321

Values are mean ± S.E-Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at **P<0.01 levels. (+,-), NS – Non-significant.

4. Discussion

Proteins are the most abundant biological macromolecules, occurring in all cells and all parts of cells. Proteins are the molecular instruments through which genetic information is expressed (Nelson and Cox, 2005). Proteins can be informally divided into three main classes, which correlated with typical tertiary structures as globular proteins, Fibrous proteins and membrane proteins. Generally, the breakdown of proteins dominates over synthesis under enhanced proteolytic activity (Murray *et al.*, 2007). Proteins being the most important organic constituents of organs, their role in the compensatory mechanism of animal can be accepted during stress conditions (Singaraju *et al.*, 1991). Protein depletion observed in the present study due to the distraction of structural proteins is evident histologically as hepatocellular membrane damage, caused by the interference of experimental compounds and their toxic metabolic intermediates (Bhushan *et al.*, 2010; Sakr *et al.*, 2003; Sakr *et al.*, 2004). The observed reduction in protein level may be due to impaired protein synthesis or their possible utilization for metabolic demands. Accumulation of toxicants in organs such as liver and kidney may lead to impaired protein synthesis (Elia *et al.*, 2011).

Amino acids are essential intermediates in the process of protein synthesis and its degradation products appear in the form of different nitrogenous substances. The oxidation pathway starts with the removal of amino group by a transaminase; the amino group is then fed into the urea cycle (Brosnana, 2000). The product of transamination is a keto acid that enters the citric acid cycle. Glucogenic amino acid can also be converted into glucose, through gluconeogenesis (Young and Ajami, 2001). The increase in the levels of free amino acid can also be attributed to the synthesis of amino acids in addition to their elevation by protein hydrolysis. A third possibility for increased amino acid level might be their increase due to transamination and deamination of keto acid (Dubale *et al.*, 1979; Jayantha *et al.*, 1983). Alkalhera *et al.* (2005) have reported the depletion of glycogen reserves of

liver in the atrazine administered animal. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis (Singh *et al.*, 1996). This increase can also be attributed to the synthesis of amino acids in addition to their elevation by protein hydrolysis (Reddy *et al.*, 1991; Prashanth and David, 2006; Anita *et al.*, 2010). The increase in protease activity under stress conditions clearly suggests that atrazine induces high protease activity which leads to the formation of higher free amino acid content which is in agreement with Patil and David (2009).

Glucose is the major energy source in the body. Glycogen is the storage form of glucose and glycogen is stored in liver. Pursuant to the present observation, level of glucose was increased in Group II at 28 days of sub-lethal dose of atrazine. The elevation of glucose levels was widely used as a secondary marker of a stress response (Toal *et al.*, 2004). Thereby, hepatic glycogen is rapidly converted into glucose and passes into systemic circulation ever-increasing the blood glucose level (Datta and Kaviraj, 2003).

Glycogen depletion in liver after toxic stress has been reported in several studies with aquatic animals (Ravinder *et al.*, 1988). Glycogen is the polymer of glucose and is known as animal starch in muscle. Glycogen is the fundamental metabolic gas in the muscle groups of majority of animals (Wittenberger, 1996). The drop in hepatic glycogen was a consequence of abruptly increased catabolism to meet higher pyrethroid-induced energy demands. Undoubtedly, the hypoxic condition is responsible for incomplete energy output through glycolysis and Krebs's cycle. Hypoxia may be responsible for necrotic lesions (Sakr *et al.*, 2004; Omotuyi *et al.*, 2006; El-Demerdash *et al.*, 2004; Manna *et al.*, 2005; Rezg *et al.*, 2007). The present observation, glycogen level was decreased in Group II. The Vitamin C acts as an electron donor for important enzymes. Moreover, the Group III (atrazine along with *Pisonia alba*) also enhance the glycogen level very slowly than the Group II.

The present consequence, level of glycogen was decreased in Group II at 28 days of

sub-lethal dose of atrazine. But the Group IV, the glycogen level was gradually increased when compared to the Group II. Because of the increases was may be the presence of valuable bioactive molecules such fatty acid, stearic acid and linolinic acid having in *Pisonia alba*. Moreover, the Group III (atrazine along with *Pisonia alba*) also improve the glycogen level very slowly than the Group IV. At the *P. alba* having the certain important medicinal properties.

The preliminary phytochemical studies of *Pisonia alba* showed the presence of Vitamin A, Vitamin C, thiamine, riboflavin, nicotinic acid (Vitamin B3), alkaloids, proteins and fats. Vitamin C is one of the four dietary antioxidants, the others being Vitamin E, Vitamin A precursor β -carotene and Selenium (Dhanasekar and Sorimuthu, 2005). The bioactive compounds present in *Pisonia alba* which may give recovery to rat in the presence of toxic stress.

5. Conclusion

The present study concludes that the treatment of *Rattus norvegicus* to sub-lethal dose of atrazine caused alterations in biochemical parameter such as protein, amino acid, glucose and glycogen in liver tissues. These alterations in treated rat suggest the operation of mechanism to cope with the toxic stress of atrazine. Results of the recovery data suggest that the *Pisonia alba* may protect on certain biochemical parameter such as protein, amino acid, glucose and glycogen is reversible in nature than the *Pisonia alba*. At the same, the *Pisonia alba* having the certain important medicinal properties.

Acknowledgement

The author is grateful thank to University Grant Commission (UGC), New Delhi, for providing financial assistance by granting major research project.

6. References

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